Cyanobacteria in space—the role of synthetic biology in the development of specialized cyanobacteria for long-term space flight

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Benefits of Cyanobacteria for NASA

- Food or food supplement
- Atmospheric O_2, CO_2 sequestration

NSCORT--
Bioregenerative Life Support, 1990s

- Excellent system for:

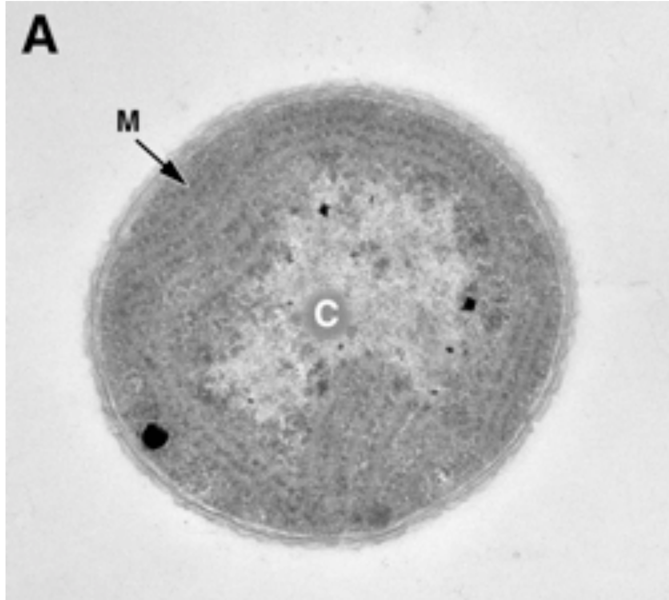
- Analysis of photosynthesis and metabolism
- Genetic diversity
- Role of gene duplication and evolution
- Production of special compounds, including biofuels
- Use of molecular biology to construct novel variants for research or for spaceflight missions.

Outline, based upon ideas in article:

A powerful toolkit for synthetic biology: over 3.8 By of Evolution
Lynn Rothschild in *BioEssays* 32:304 (2010)

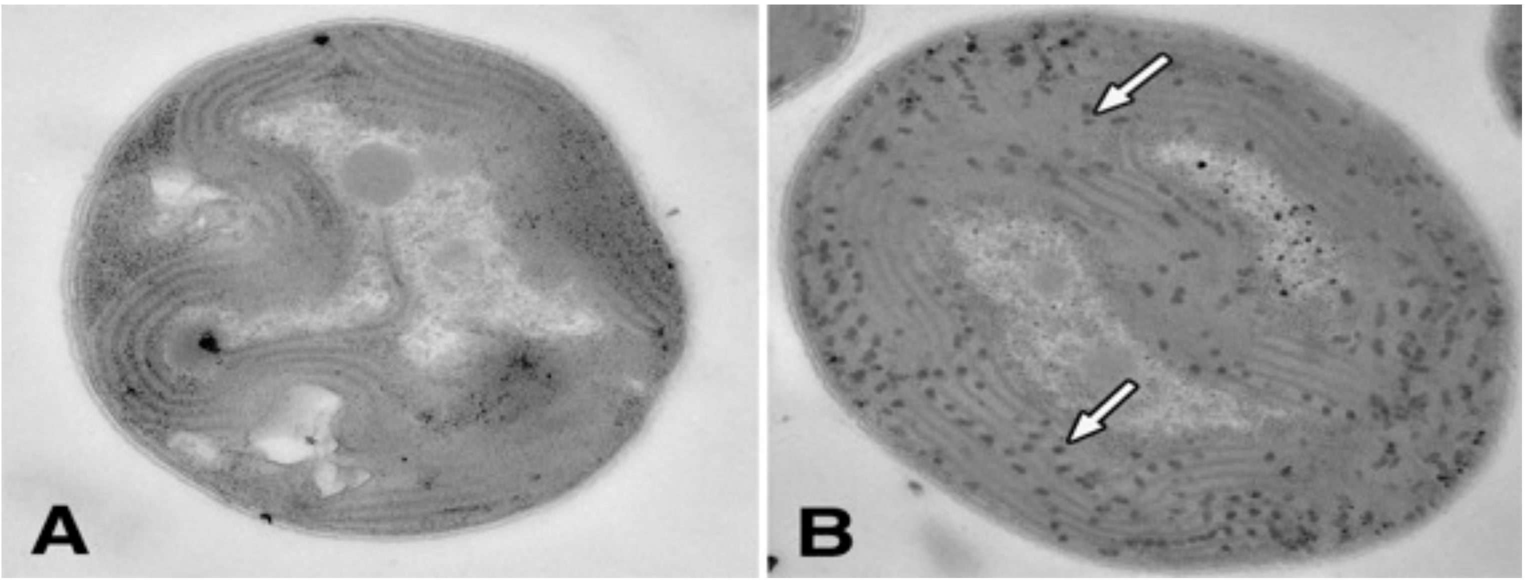
- Genomic plasticity of cyanobacteria
- Gene duplication
- Regulatory changes
- Ability to switch PSII into different modes of replacement
- Environmental plasticity--growth under anaerobic conditions
- Metabolic plasticity--Production of materials—
  for food, for atmosphere, for energy

Synechocystis sp. PCC 6803

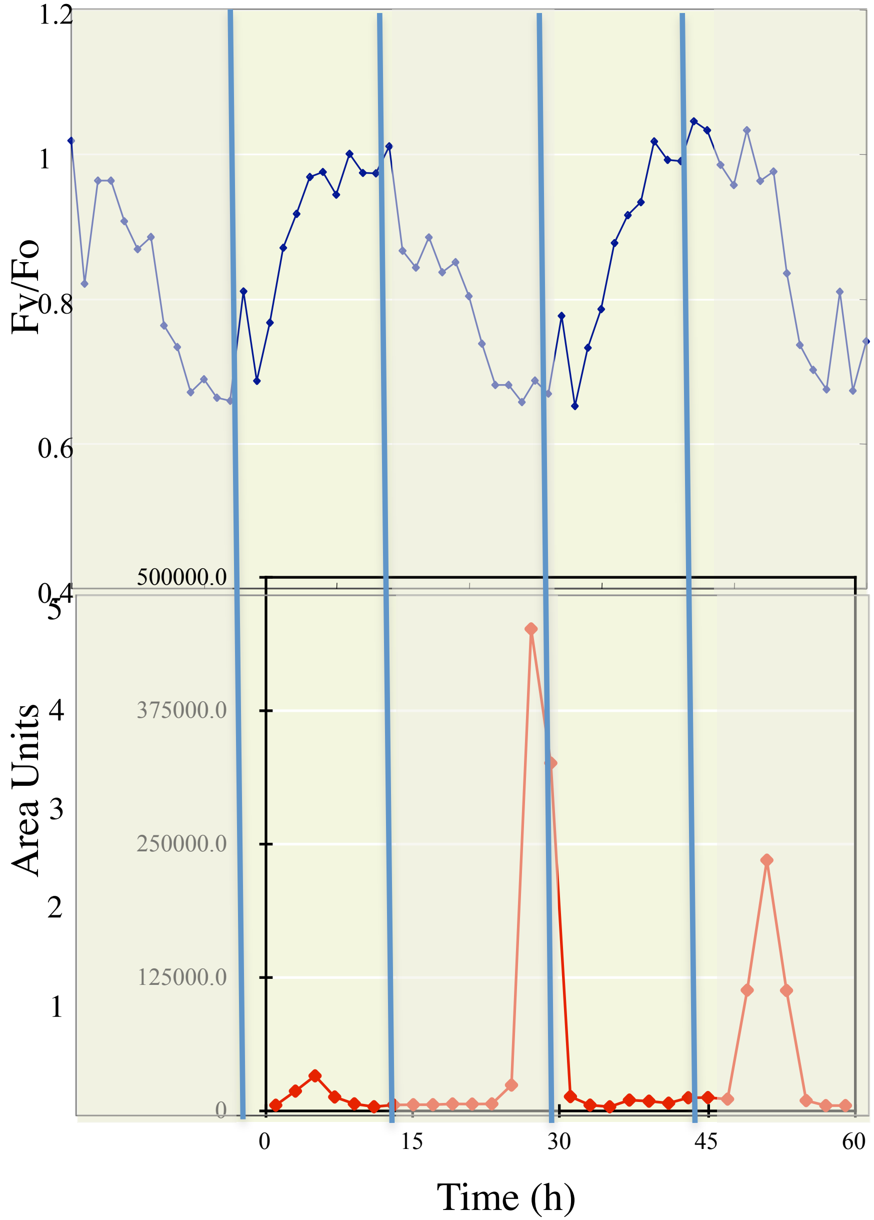


PA-grown Δ*hik8*

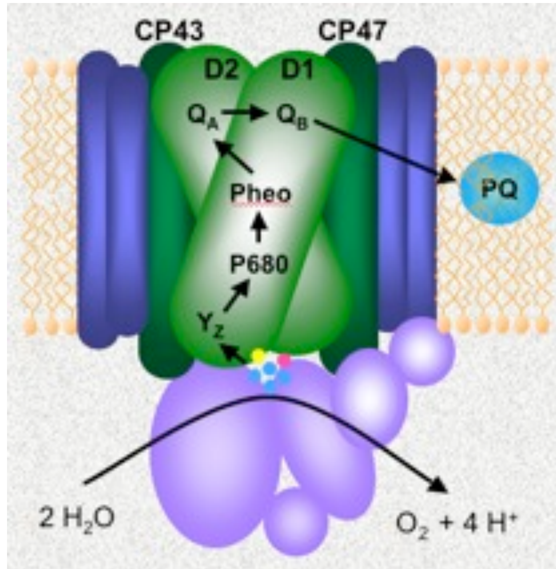
PA-grown WT



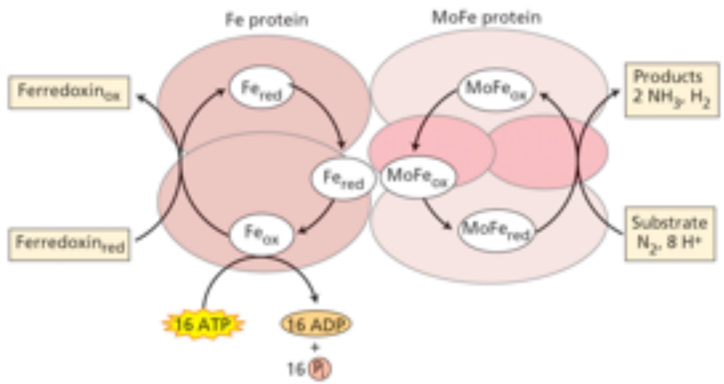
Diurnal Rhythms in *Cyanothece*

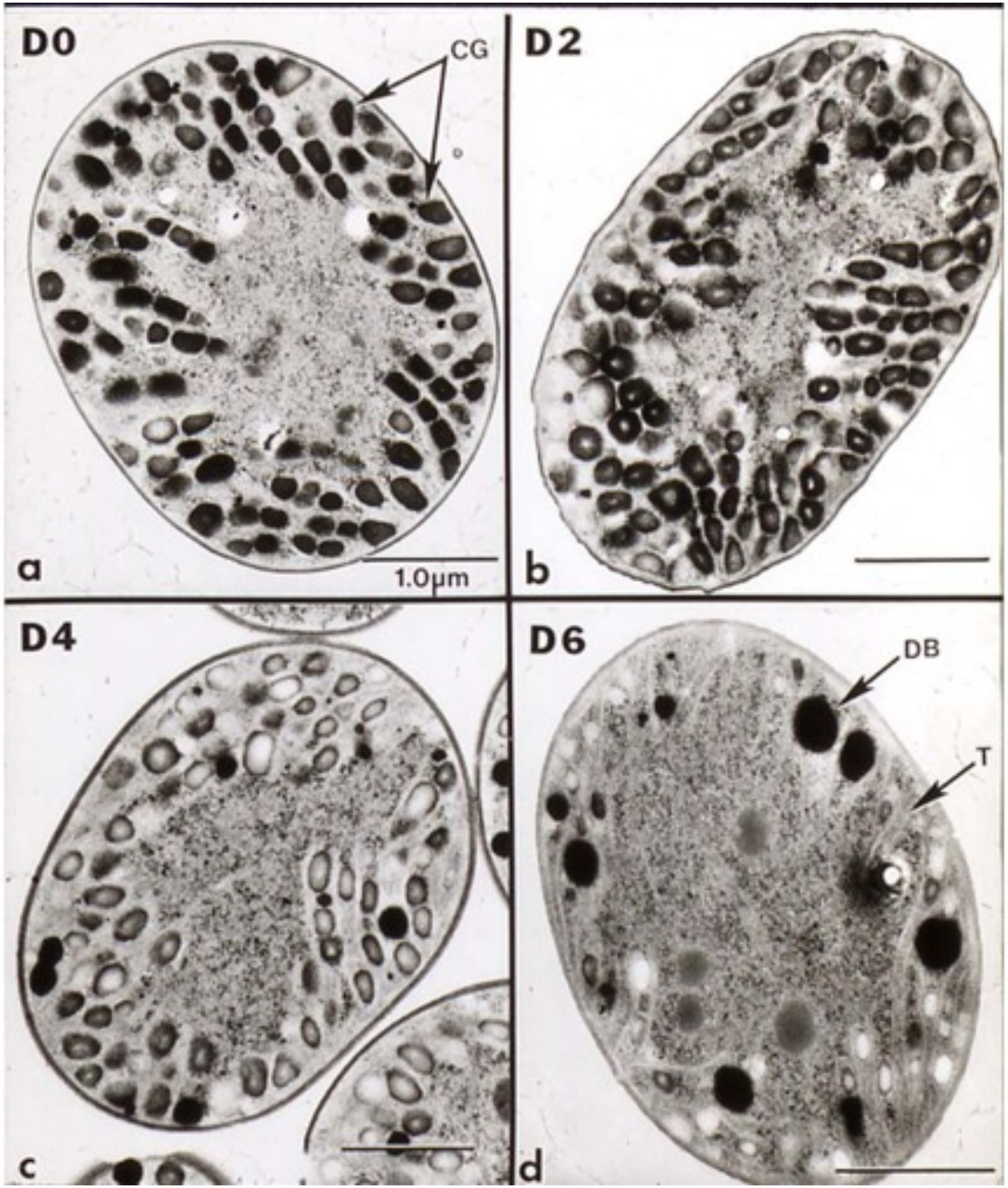


Photosynthesis

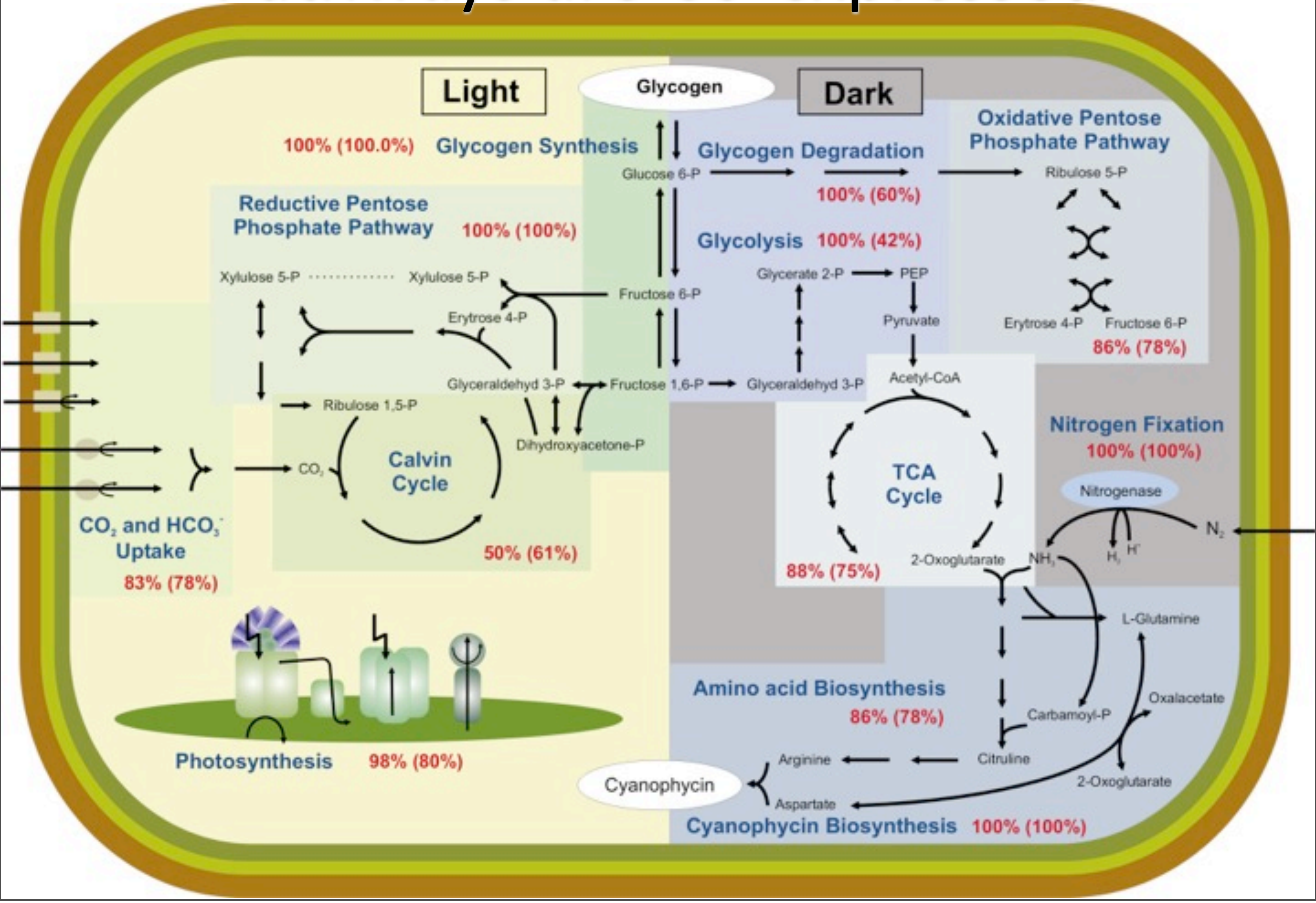


Nitrogen fixation

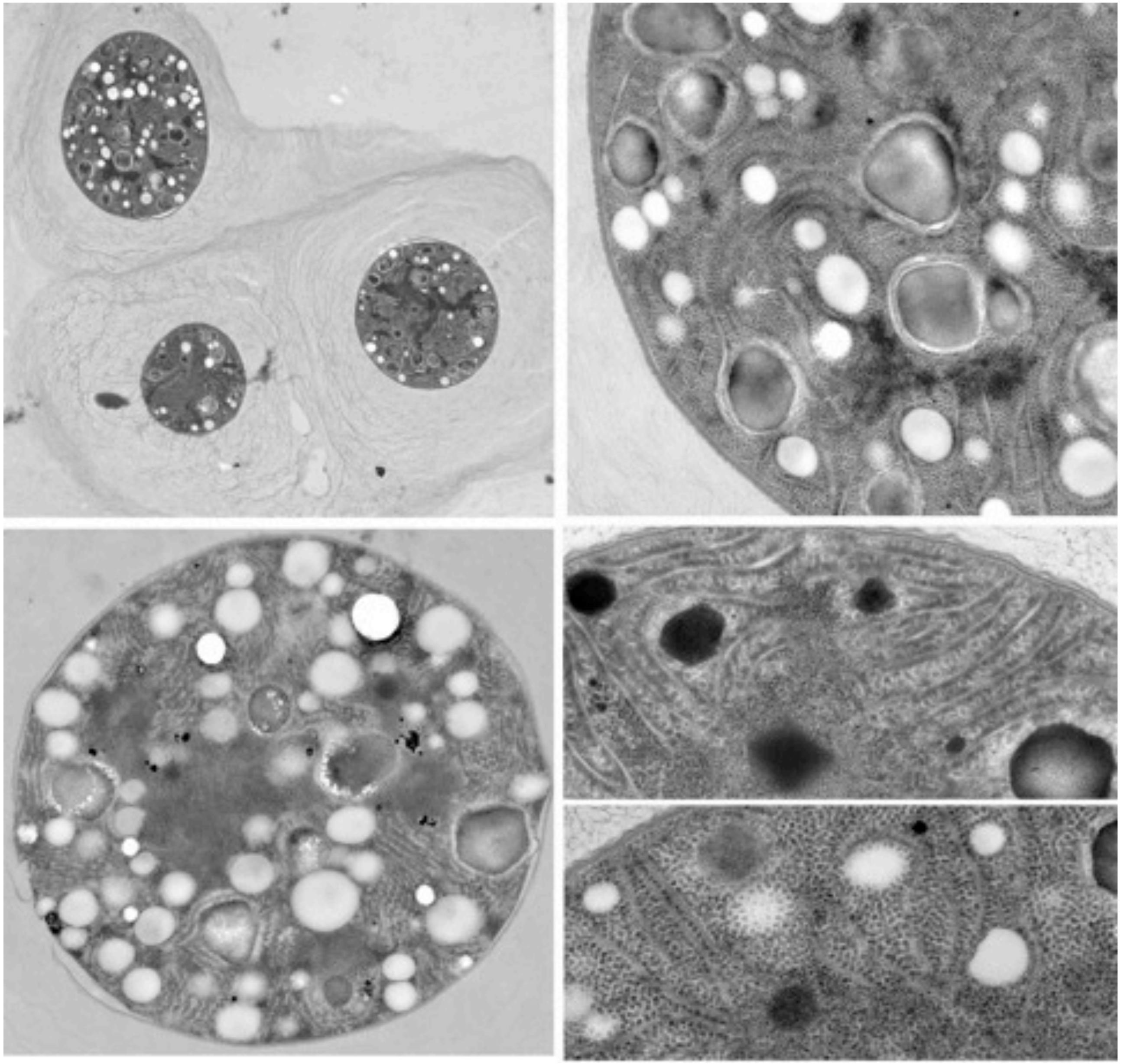



D0
CG
a
1.0μm
D2
b
D4
c
D6
DB
T
d

Pathways are Co-expressed



Cyanothece sp. PCC 7822--the granule expert



Cyanobacteria grow under a wide range of environmental conditions

Parameter	Conditions
Temperature	Low to High (~0C to ~100C)
Hydration	Desert to Oceans
Salt	Low to High
Nutrients (N, C, S, P)	Low to High
Light Intensity	Low to High
O_2 levels	aerobic (20%) to anaerobic (≤0.1%)

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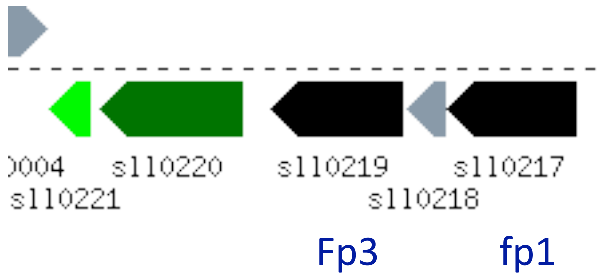
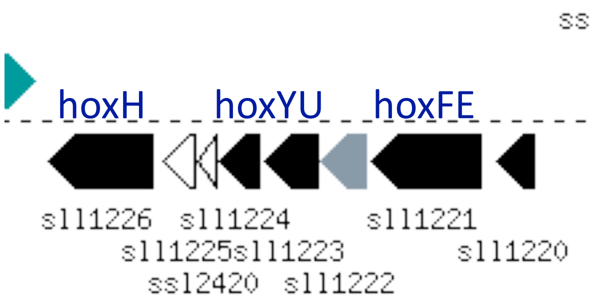
Gene clusters up-regulated during anaerobiosis in *Synechocystis* sp. PCC 6803 (doesn't fix N_2)





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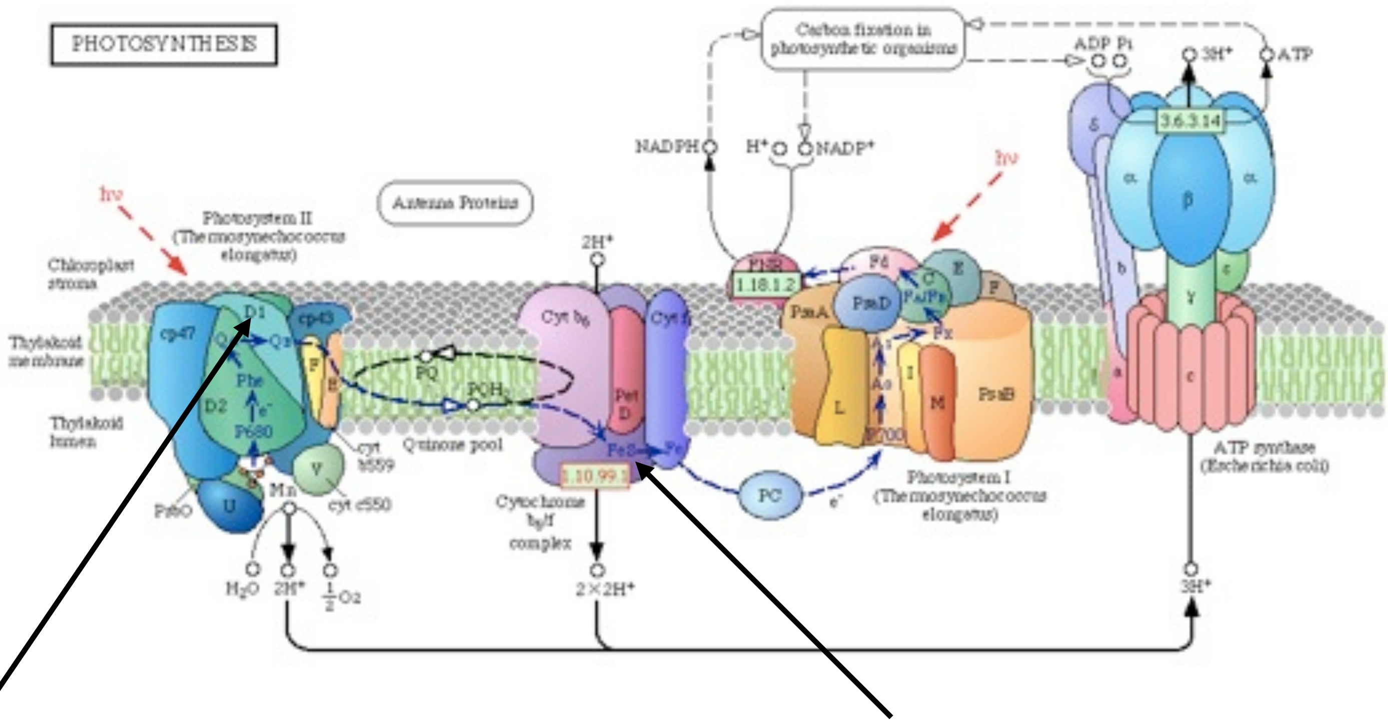
psbA1 and petC2 encode components of the photosynthetic electron transport chain



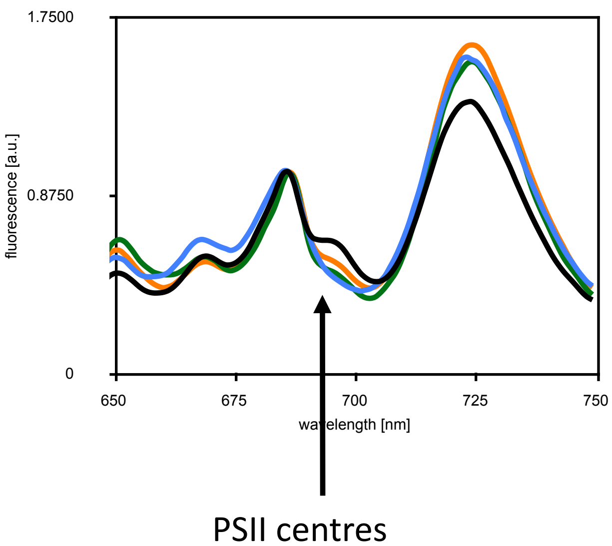
psbA1 encodes alternate D1 (D1')

petC2 encodes Rieske iron-sulfur protein

psbA1-encoded D1’ is assembled into functional PSII centers

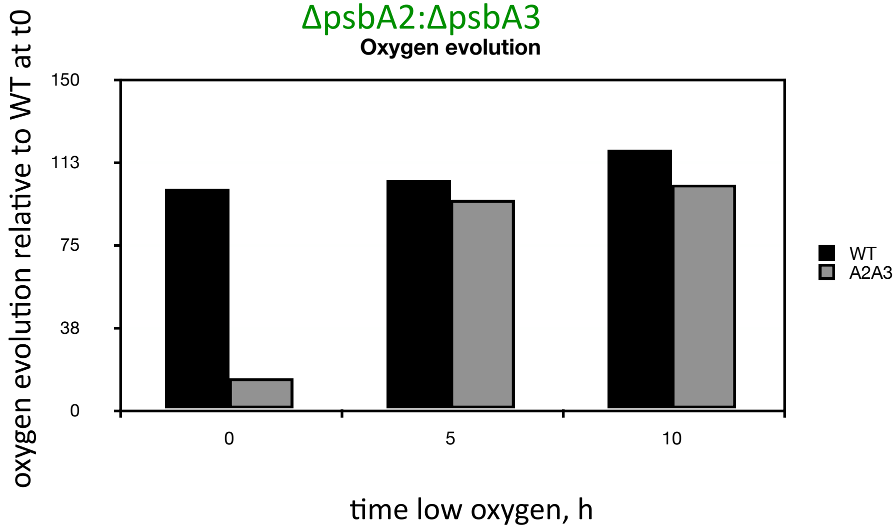
77 K fluorescence
excitation at 440 nm

Wild type
ΔpsbA2:ΔpsbA3 t0
ΔpsbA2:ΔpsbA3 t5
ΔpsbA2:ΔpsbA3 t10



Oxygen evolution

Wild type
ΔpsbA2:ΔpsbA3



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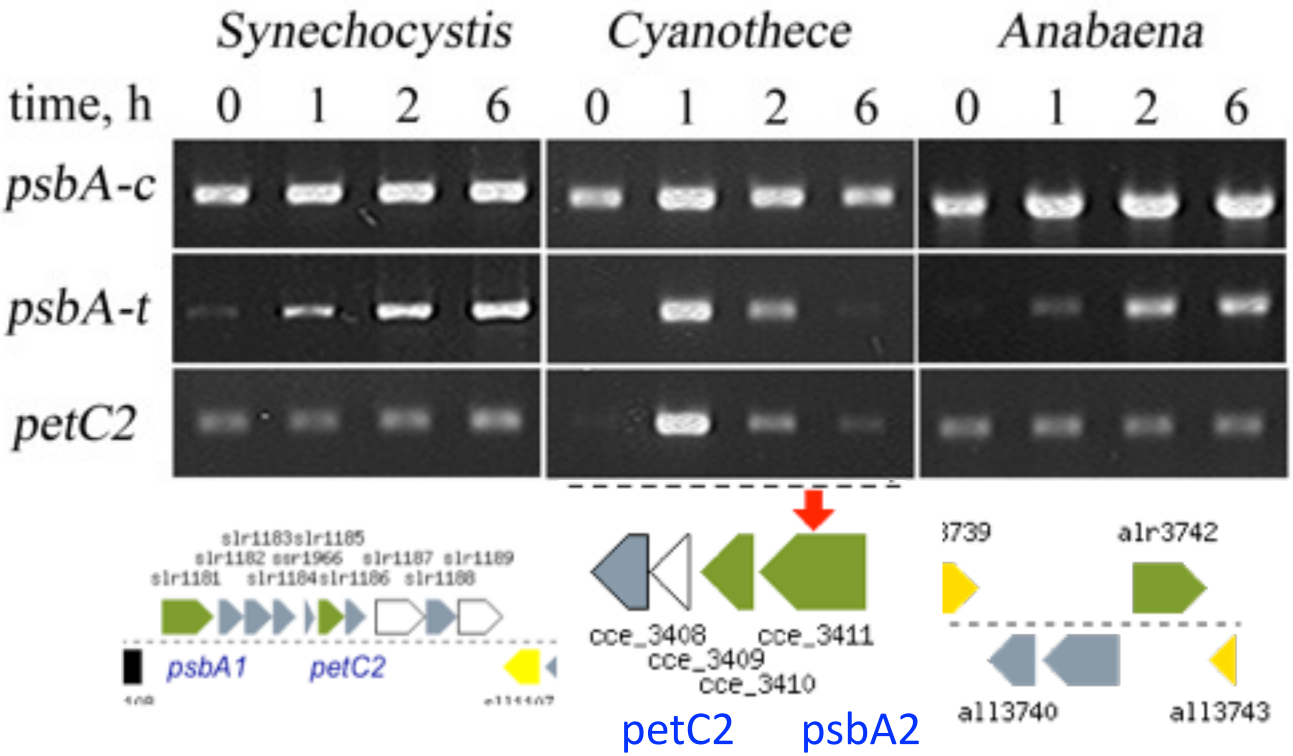
Anaerobic induction of psbA and petC2 genes in 3 cyanobacterial strains



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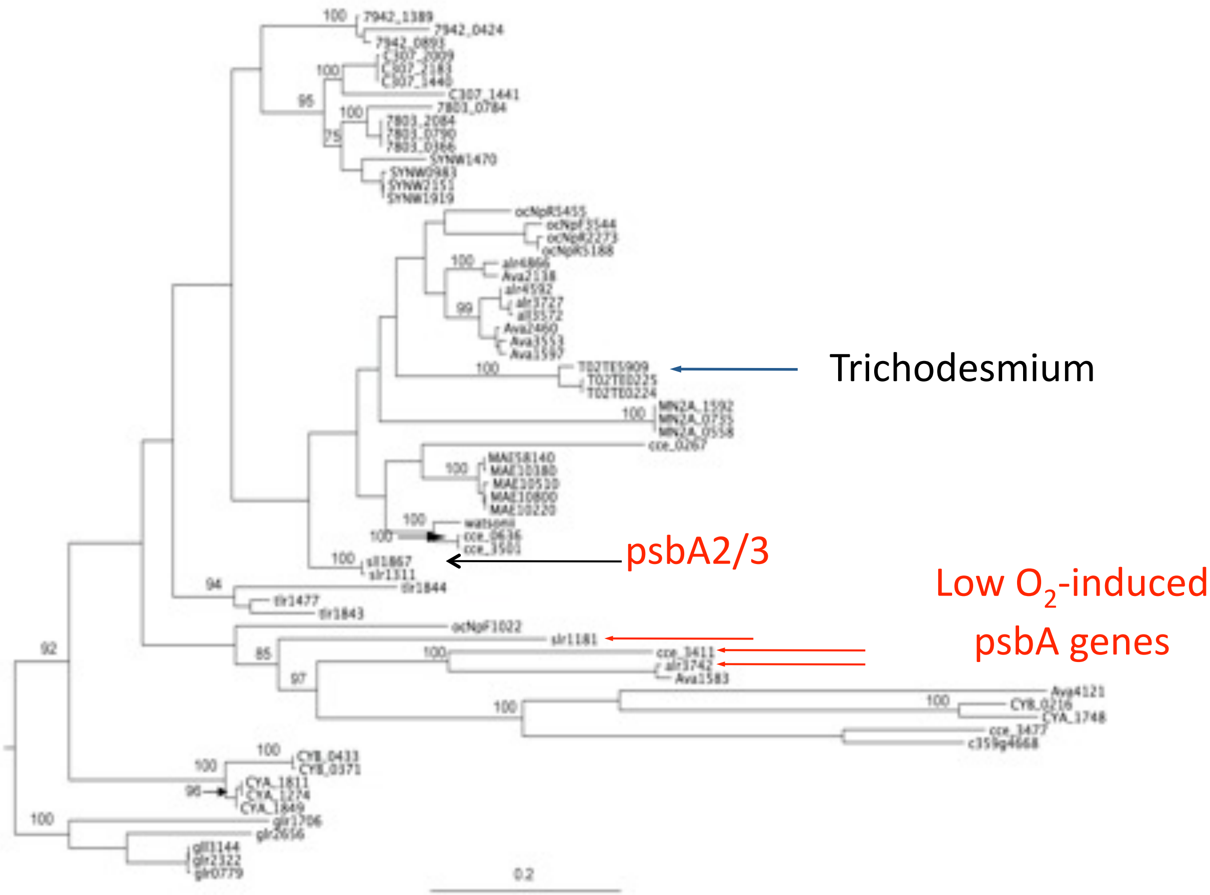
psbA genes from 17 cyanobacterial strains



Maximum likelihood

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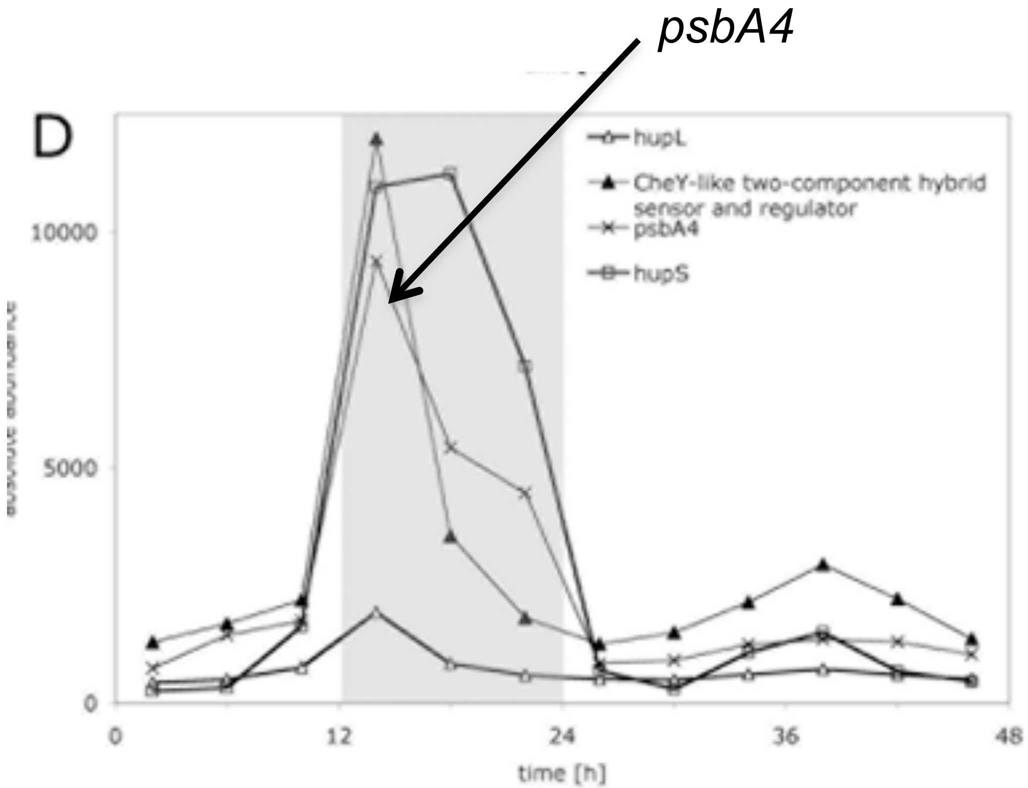
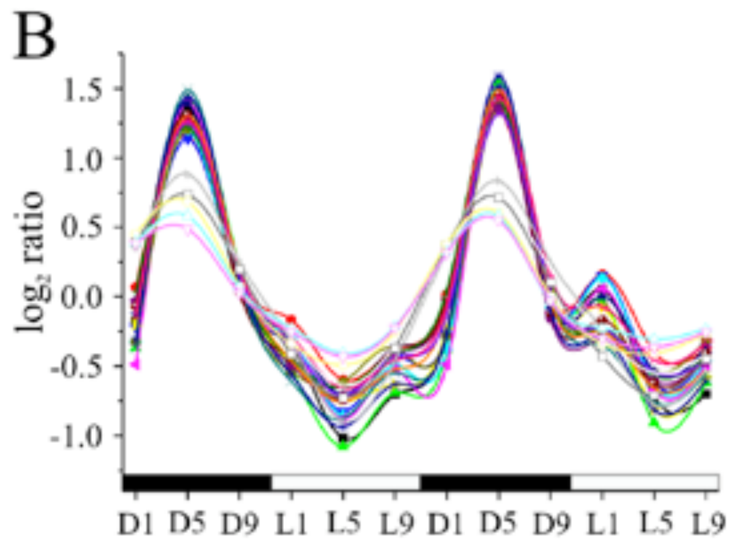
Transcription in *Cyanothece* 51142 during growth under N_2-fixing conditions in Light-Dark. The *psbA4* gene (left panel) is transcribed strongly in the dark, the same time as the nitrogenase genes (right). This gene has mutations in all the amino acids needed to bind the Mn_4-Ca^{+2} complex that is essential for O_2 evolution.





CONCLUSION: cyanobacteria can alter PSII composition and function based on environmental conditions and cellular requirements.

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Why do cyanobacteria have multiple *psbA* genes?

The D1 protein needs to be replaced because it is damaged during photosynthetic electron transport.

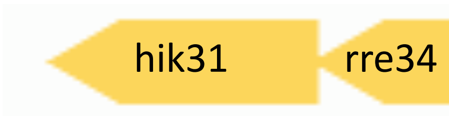
The cell has the ability to insert different D1 proteins depending on environmental conditions—e.g., PsbA2 under anaerobic conditions or PsbA4 under N_2-fixing conditions in *Cyanothece*.

The anaerobic-inducible *psbA* genes, like *psbA1* in *Synechocystis*, is functional, whereas *psbA4* in *Cyanothece* is probably not fully functional. Yet, they allow the repair of PSII and keep this expensive complex intact.

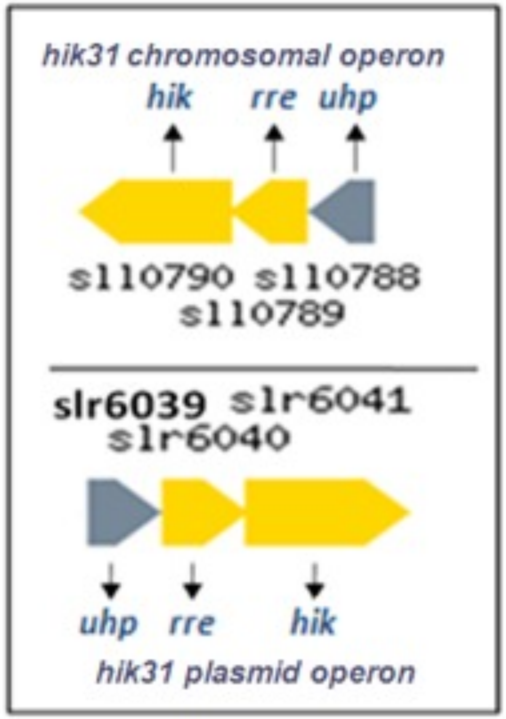
Such plasticity has many implications for NASA's many missions—e.g., evolution and search for life, long-term space flight and planetary colonization.

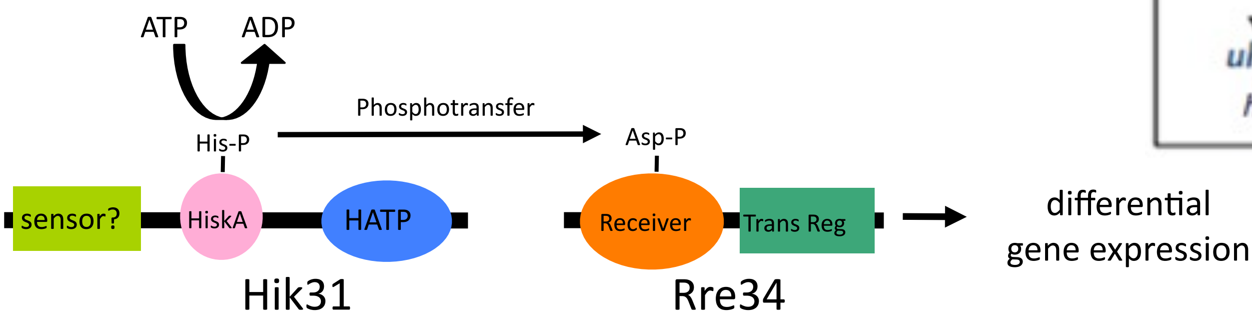
Genes encoding a two component system were up-regulated

- transcripts encoding a histidine kinase (Hik31) and a response regulator (Rre34) are up-regulated under low oxygen conditions



	hik31	rre34
t1/t0	3.1	2.0
t2/t0	2.3	2.1
t6/t0	1.2	1.5





czc genes slr6042/slr6043neighbors and strongly induced under low O_2

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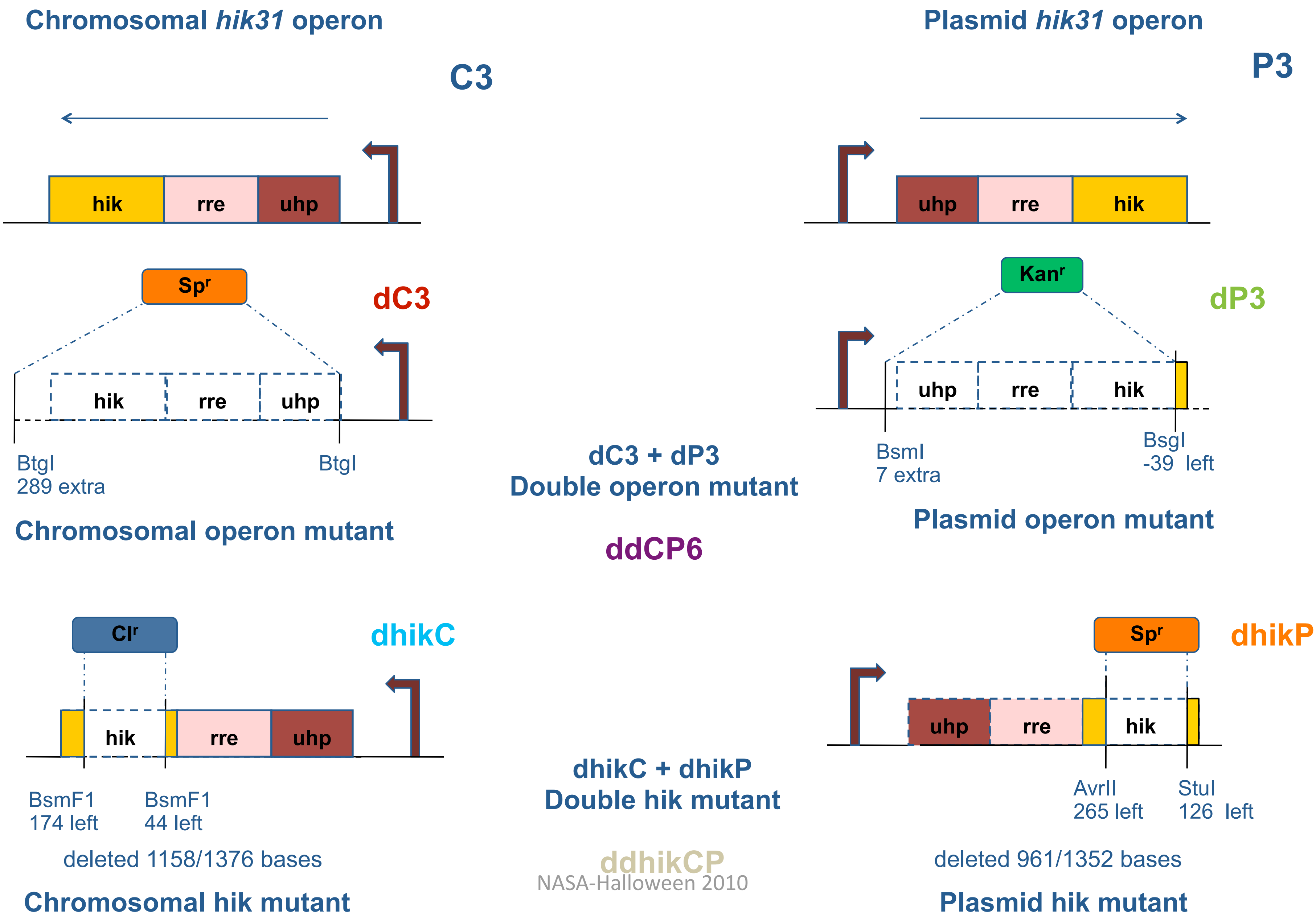
Deletion Mutant construction

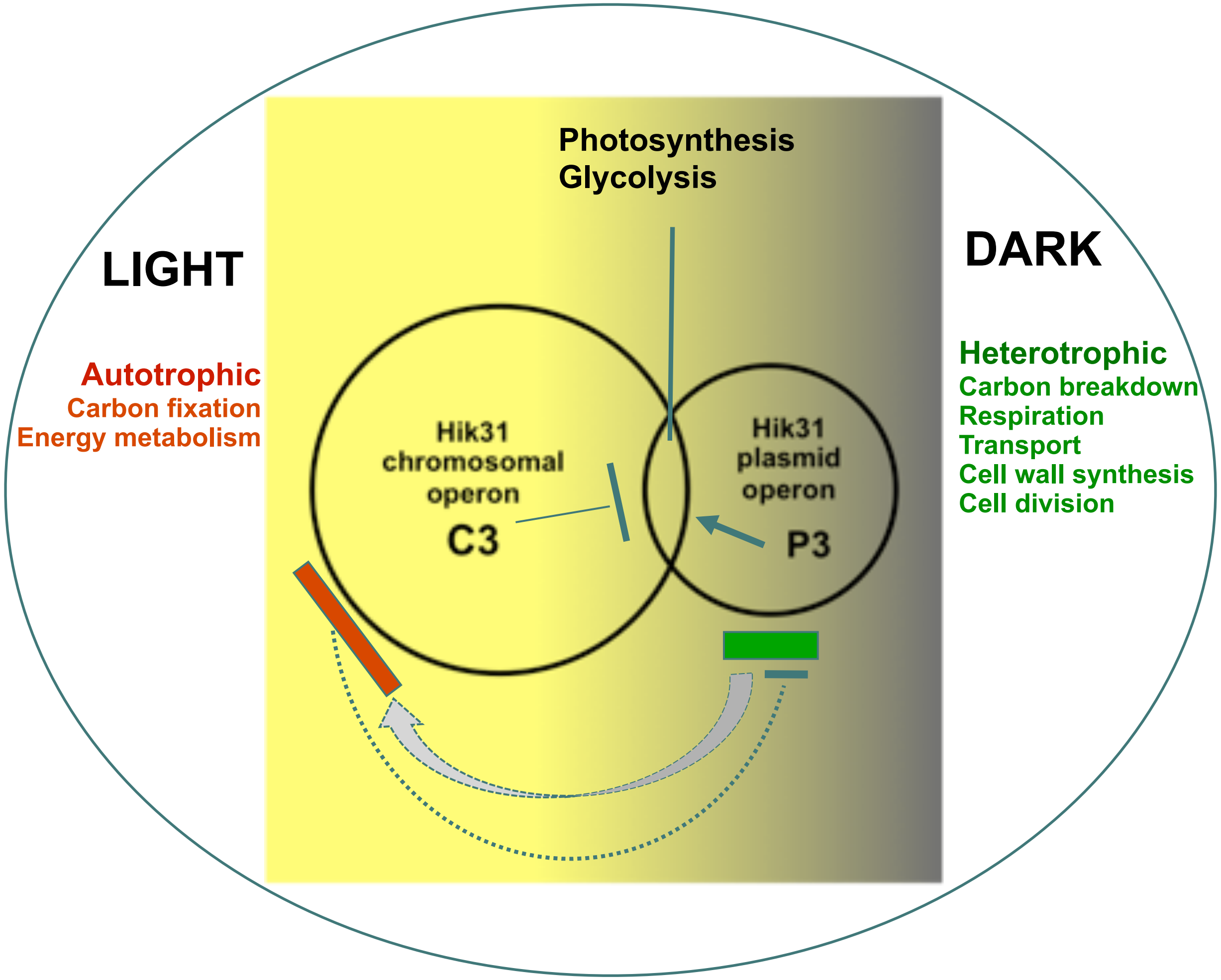


Growth of *Synechocystis* sp. PCC 6803 wild type and *hik31* mutants with air bubbling for 48 h (aerobic) followed by 99.9% N_2 and 0.1% CO_2 (low O_2) for a subsequent 48h

Strain	cells/ml ($x10^7$)			Low O_2 growth increase
	0 h	48 h air	96 h low O_2	
WT	1.2	3.5	5.3	1.5x
Δhikc[b]	0.9	3.8	7.6	2.0x
Δhikp[c]	0.9	4.3	7.2	1.7x
Δhikcp[d]	1.5	4.8	11.7	2.4x

Total of 12 experiments, with S.D. ±15%

Current Model for Hik31 regulation during light-dark growth with and without glucose



Both copies regulate separate and common metabolic processes in light and dark.
Regulatory relationship between the two operons affected by: Light, Dark and Glucose
Anaerobic growth: The lack of HikC and HikP enhances growth.

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Global gene expression in Δ*hik31* under low oxygen conditions

- compared to wild type the Δ*hik31* strain showed up-regulation of genes including those associated with growth
  - ribosomal proteins
  - ATP synthase
- suggests that Hik31 is involved in down-regulating gene expression under low oxygen conditions
- Could current function for the 2 Hik31 systems have developed once cells had begun to grow under aerobic conditions?

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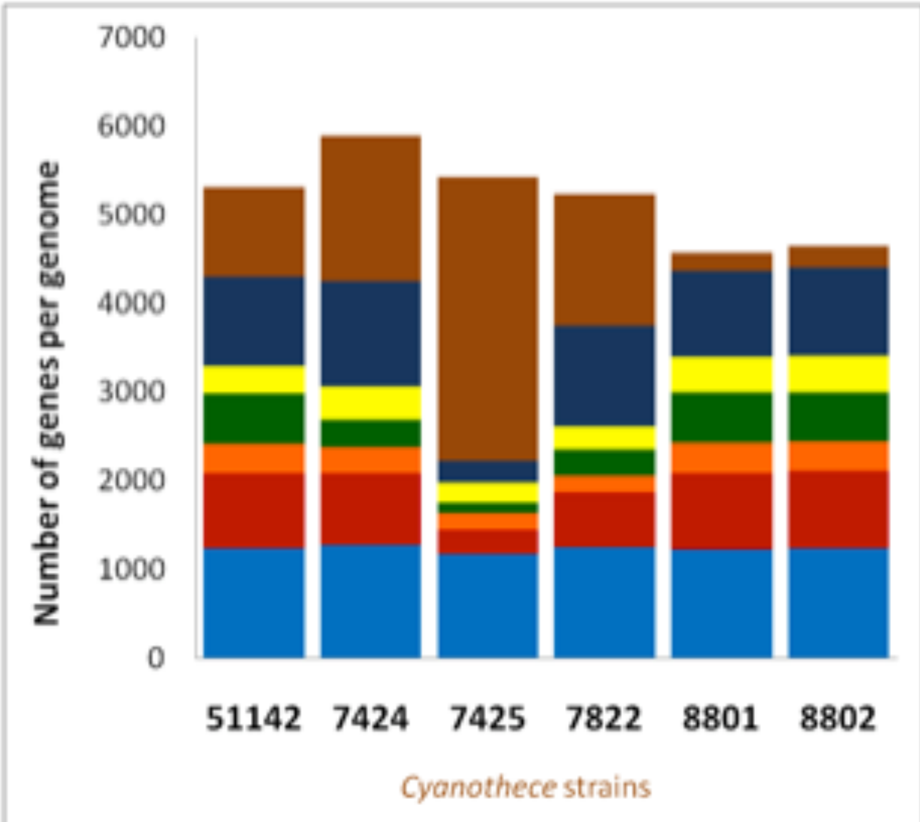
Genome sequence data on 6 strains of *Cyanothece*

Strain	PCC 7424	PCC 7425	PCC 7822	PCC 8801	PCC 8802	ATCC 51142
Mixotrophic Growth	Yes	Yes	Yes	Yes	Yes	Yes
Phycoerythrin	Yes	No	Yes	Yes	No	No
Genome-Circular Chromosome - Linear Chromosome - Linear Chromosome	6.4 MB - -	5.7 Mb - -	6.1 Mb 0.88 Mb 0.47 Mb	4.6 Mb - -	4.7 Mb - -	5.5 Mb - 0.43 Mb
Gene Number	6107	5574	6646	4436	4681	5356
COG Genes	3432	3345	3006	2705	2866	3056
Carbohydrate Metabolism	176	178	159	136	145	162
Energy Metabolism	218	203	189	187	190	213

The genomes were sequenced at the DOE-Joint Genome Institute.
The strains can utilize external carbon sources indicating their metabolic flexibility.
The genomes reflect differences in metabolic properties of the strains.

Shared and unique genes in the genomes of *Cyanothece*

Homologous genes within the *Cyanothece* genomes were identified by using the protein blast algorithm from NCBI. About 1200 genes are shared among all genomes. *Cyanothece* 7425 has the largest number of unique genes. Color coding for each category is as follows : light blue - genes shared by all seven genomes, red - genes shared by 6 genomes, orange - genes shared by 5 genomes, green – genes shared by 4 genomes, yellow – genes shared by 3 genomes, dark blue – genes shared by 2 genomes and brown represents unique gene in each strain.



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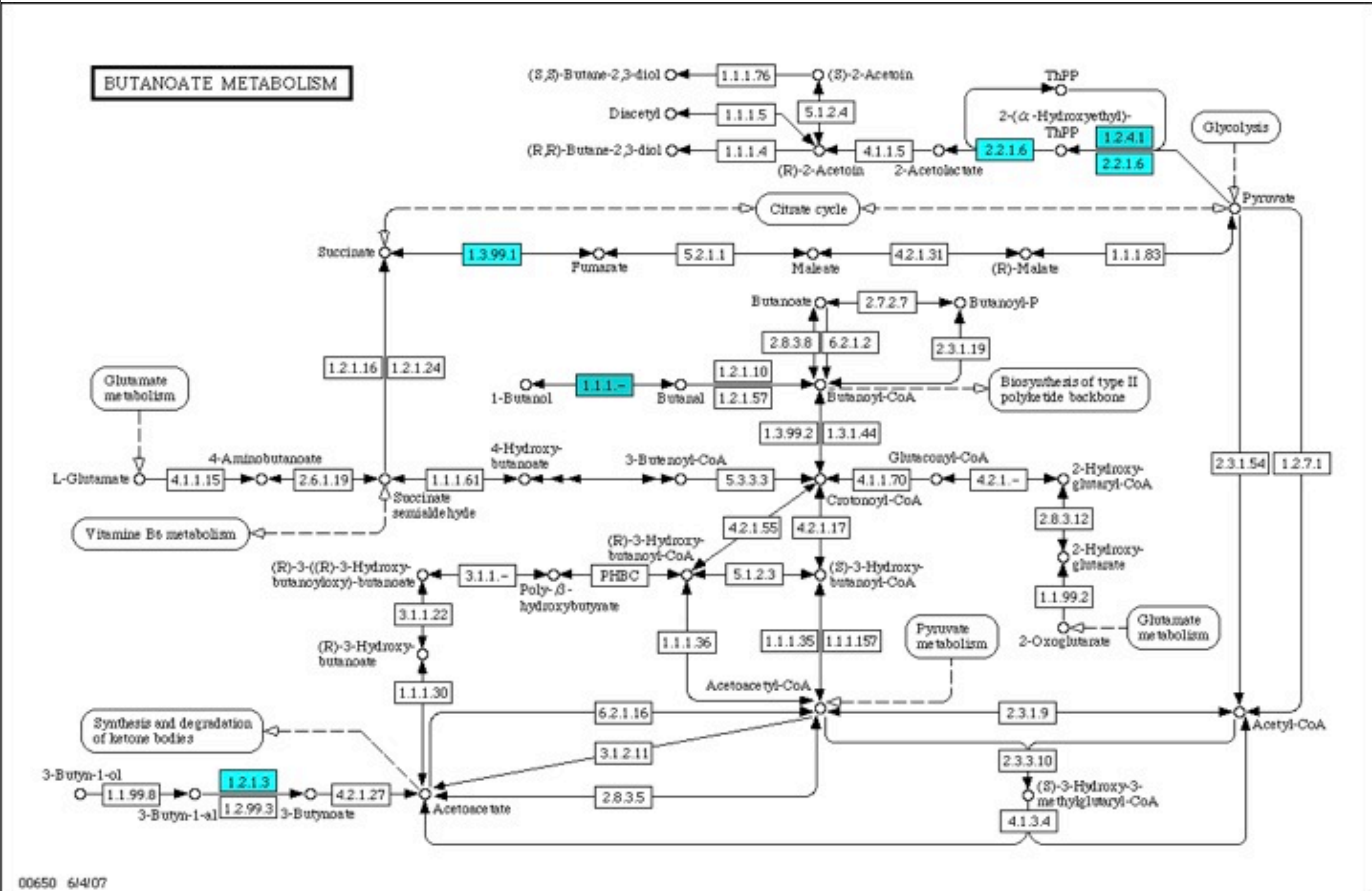
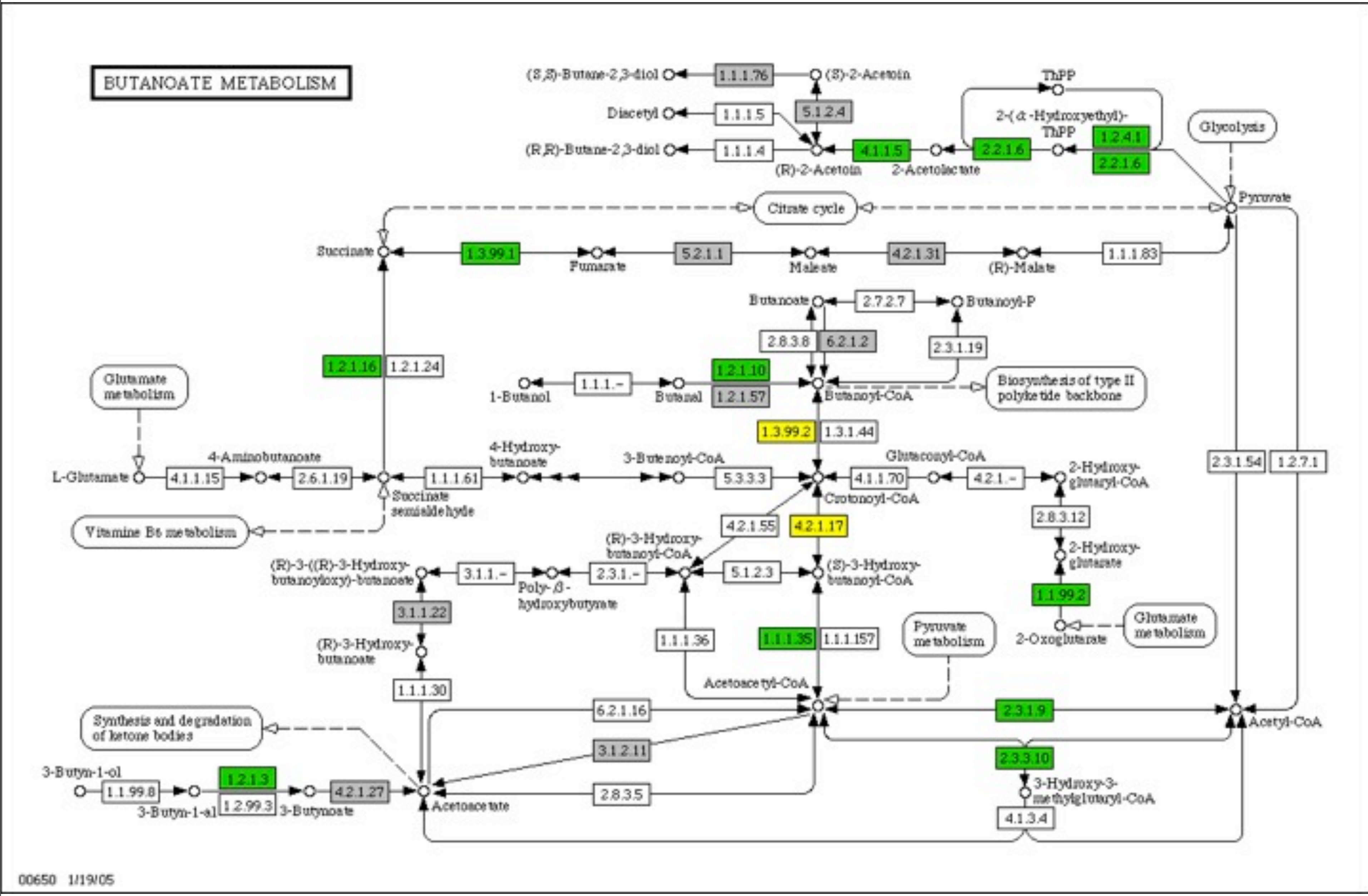
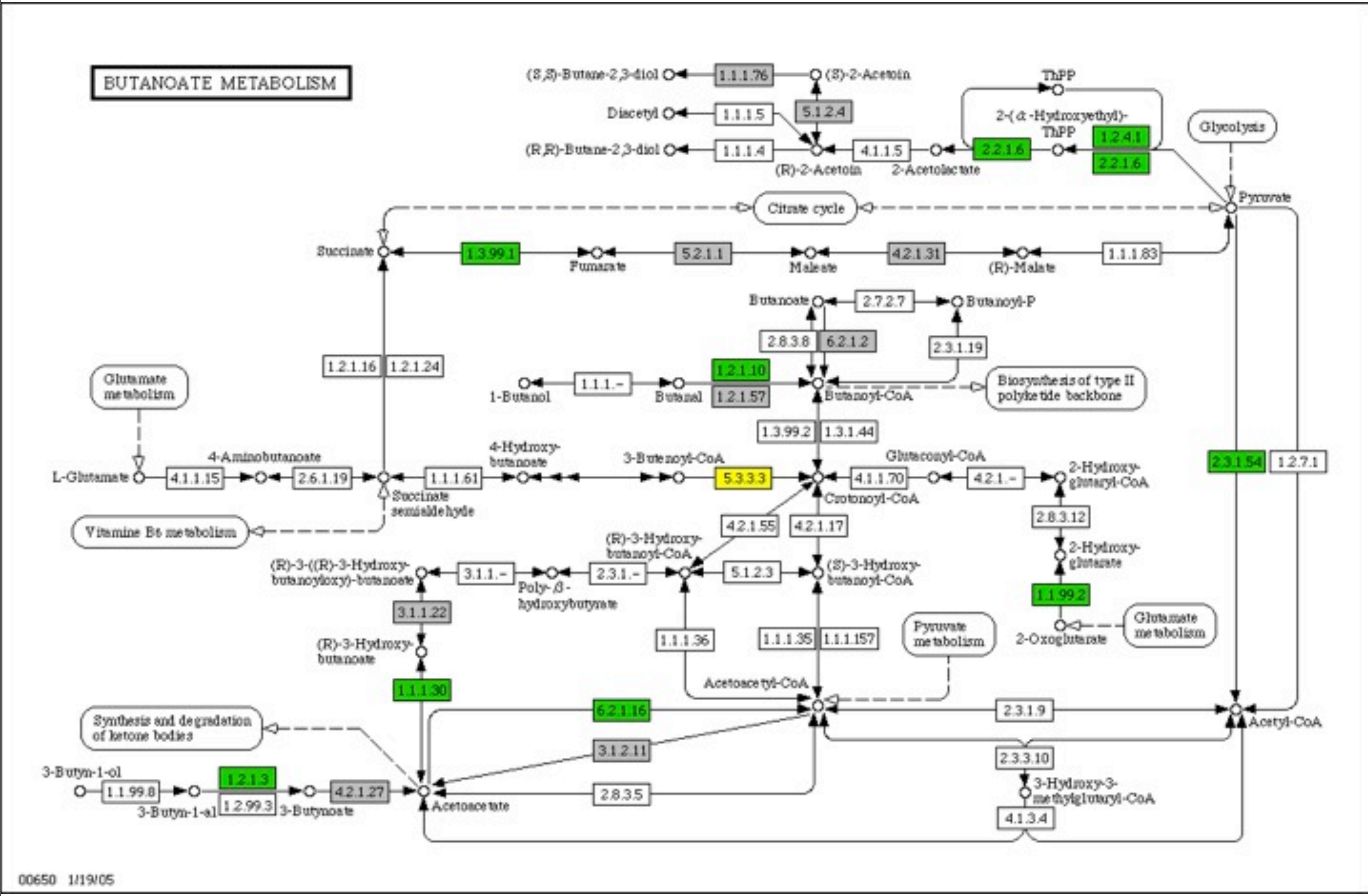
Cyanothece sp. ATCC 51142--Gulf Coast



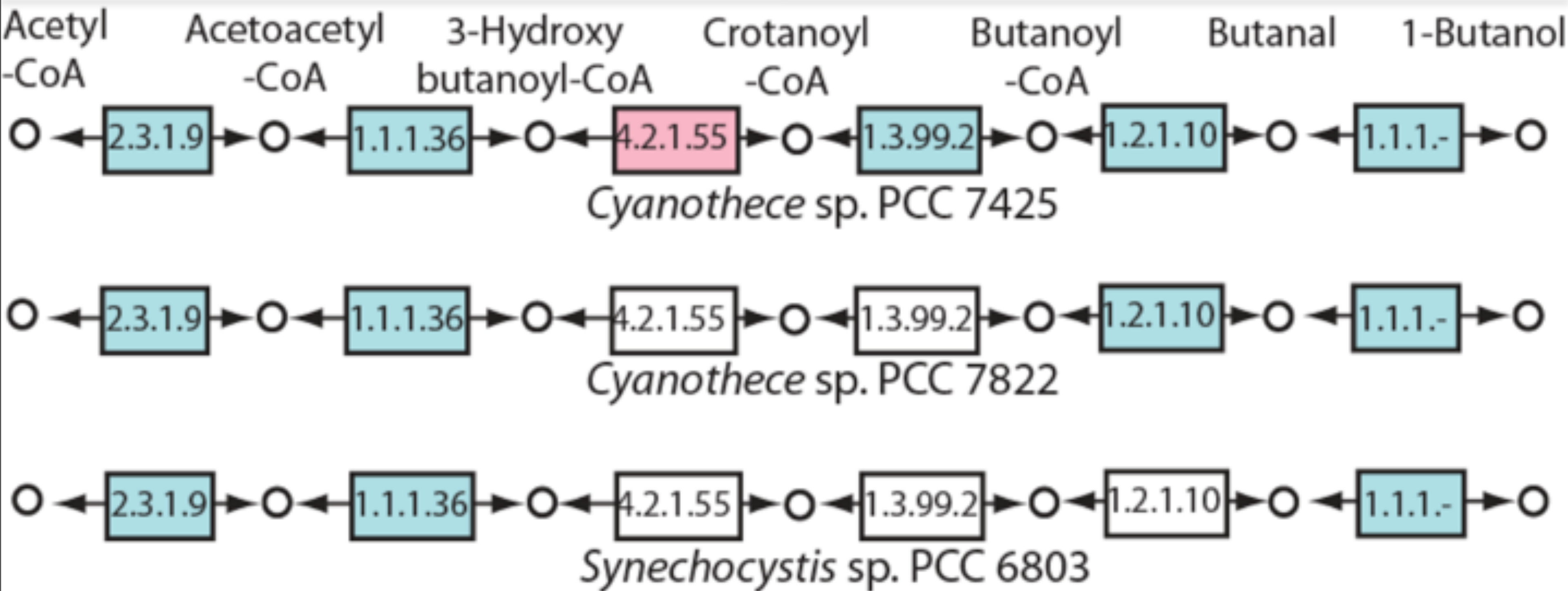
Cyanothece sp. PCC 7425-rice fields



Cyanothece sp. PCC 7822-rice fields

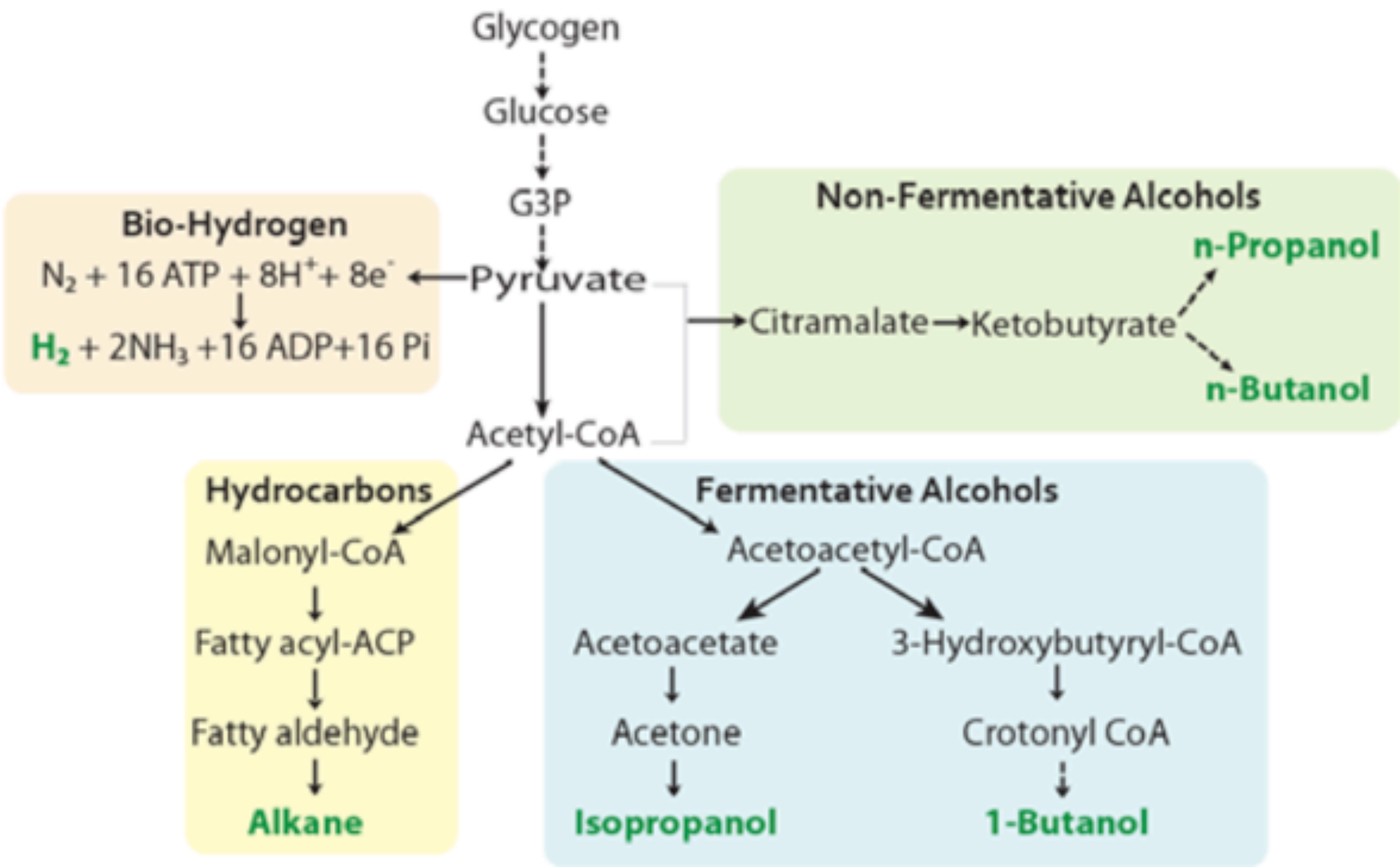


The fermentative butanol synthesis pathway in *Cyanothece and Synechocystis*



Cyanothece 7425 has all the genes involved in the pathway. White represents missing genes, blue represents genes present, pink represents a putative 3-hydroxbutyryl-CoA dehydratase gene with an enoyl-CoA hydratase motif (functional motif of 3-hydroxbutyryl-CoA dehydratase).

Metabolic pathways in *Cyanothece* leading to the fuel molecules of interest



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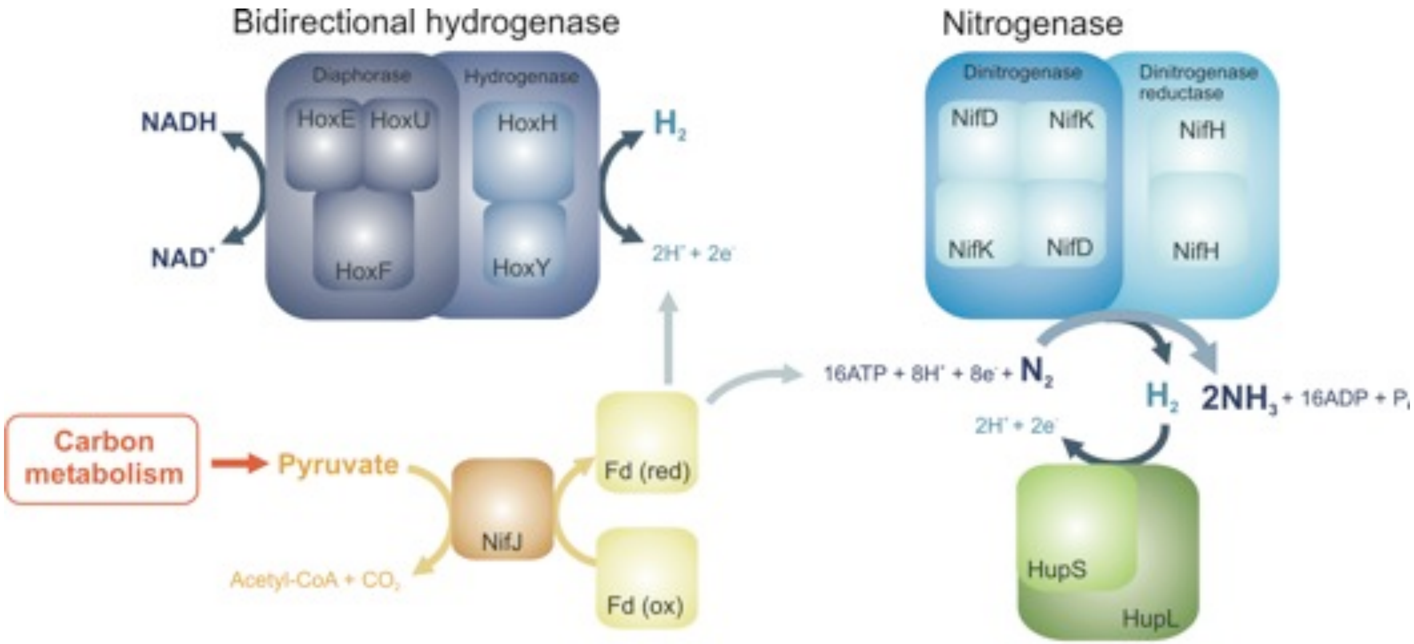
Hydrogen production



Hydrogen can be produced by Nitrogenase and Hydrogenase enzyme systems

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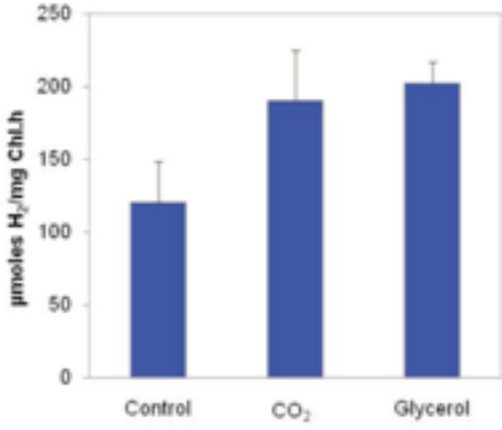
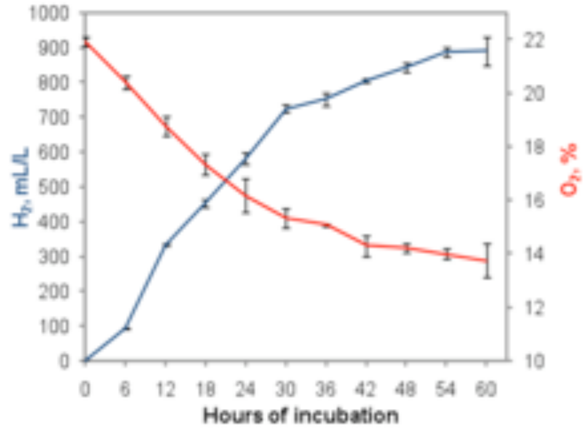
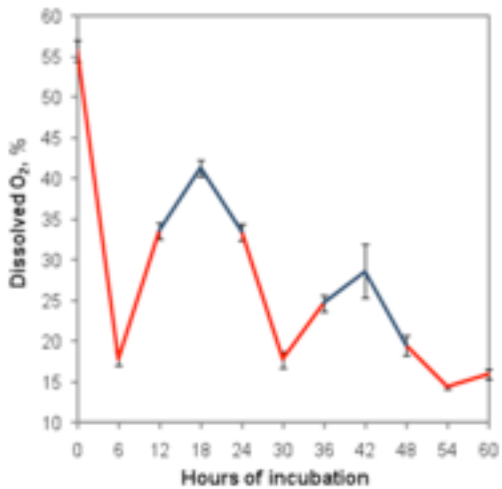
Cyanothece sp. ATCC 51142 produces lots of H_2







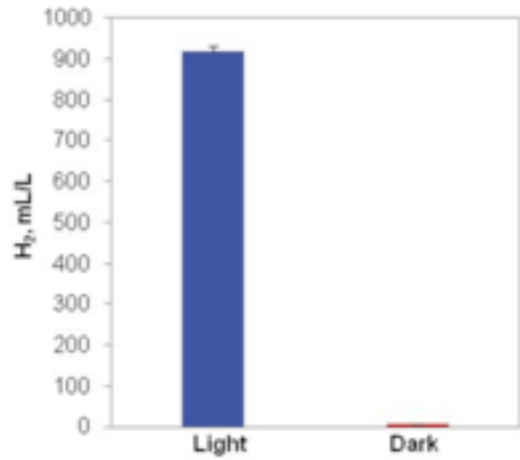


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Table 2 – Improvements in the rates of H_2 production by physiological and genetic modifications

Strain	Specific rates of H_2 production		Conditions
	(µmoles/mg protein.h)	(µmoles/mg Chl.h)	
***Cyanothece* 51142**	3.5	373	WT, photoautotrophic growth under ambient CO_2 concentrations, argon incubation
***Anabaena* 29413 (PK84 mutant)**	-	167.6	*hup*⁻ mutant[1], 2% CO_2, argon incubation[38]
***Synechocystis* 6803 (M55 mutant)**	-	5^{Δ} $^{\int}6$	*ndhB*⁻ mutant[1], glucose, glucose oxidase, sulfur deprivation, argon incubation[43]
C. reinhardtii	-	$16.26^{*\Delta}$ $5.8^{\#}$	WT, photomixotrophic growth, sulfur deprivation, anaerobic incubation[44,45]
R. palustris	3.6	-	Mutations in *hup* and *nifA*, organic carbon sources, anaerobic growth and anaerobic incubation[25]

*The rates were calculated using information from references 44 and 45

$^{\Delta}$ Initial rates of H_2 production, $^{\int}$Rates last for > 25 min, $^{\#}$ Rates last for >55 hours

Conclusions

General

- High throughput data consistent with previous work
- Enables analysis for many metabolic processes

Hydrogen production

- All *Cyanothece* spp. can produce reasonable levels of H_2
- Nitrogenase produces far more than hydrogenase

Key factors:

- Protect nitrogenase from oxygen—*in vivo*, Mehler reaction, respiration and peroxiredoxins critical; argon during incubation
- Energy—from photosynthesis; PSI cyclic sufficient and stored carbohydrate is helpful
- If nitrogenase protected from oxygen, then can occur in LL

Molecular tools exist for manipulations in both *Synechococystis* and *Cyanothece*

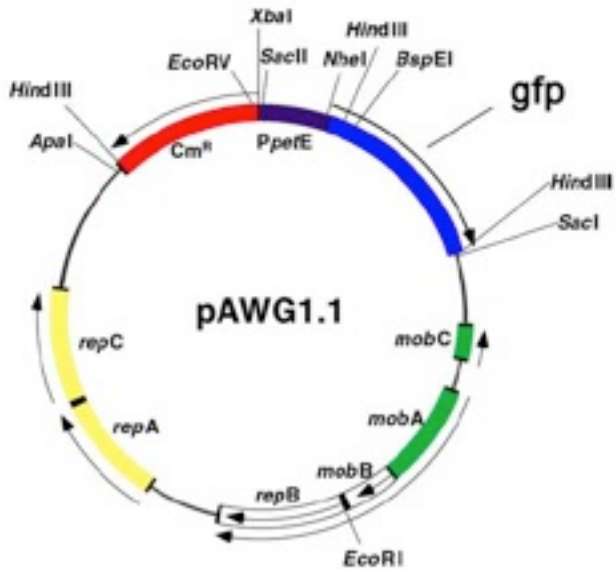

Figure 1-8: Plasmid map of the broad-host-range plasmid pAWG1.1 (Hörnemann, 2000).

Genetics—gene exchange can be generated by:

- Transformation
- Electroporation
- Conjugation

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Cyanothece core genome (1639 Genes, 966 KO's)